Peer Review File
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Reviewer A

Comment 1: the analysis to compare pediatric patients with and without comorbidities, the focus is longitudinal changes, and the clinical research design is a prospective comparative cohort study. All these information should be indicated in the title.

Reply 1: Thank you for the observation, we took your comments into account, and we have added those points in the title,

Changes in the text: We added this in the title: A prospective comparative cohort study.

Comment 2: Second, the abstract needs some revisions. The background did not indicate the clinical significance of observing the longitudinal changes in IgG levels of SARS-COV-2, as well as the focus on the differences between those with and without comorbidities. The methods did not describe the inclusion criteria, the assessment of baseline clinical characteristics including the diagnoses of comorbidities, the measurements of IgG levels, and follow up procedures.

Reply 2: thank you very much for your observation, we made a new abtract with the new information of the revision and We take into account your observations of the missing information.

Changes in the text: Background: More than two years after the start of the pandemic caused by the SARS-COV-2 virus and the disease it produces called COVID-19, there is a great lack of information. The presence of IgG may be related to disease severity and some studies in have associated IgG levels w with severe disease compared with patients with mild or moderate disease, patients with comorbidities could develop more severe infection, however, longitudinal follow-up studies evaluating the dynamics of humoral response in the pediatric population are scarce. The objective of this study is to describe the behavior of memory immunoglobulins (IgG) in pediatric patients and to know if there is a difference between patients with comorbidities. Methods: a prospective comparative cohort study. The study included all the subjects with confirmatory test for SARS-CoV-2 from 1 month to 17 years 11 months, a documented follow-up of the disease's evolution and measurement of IgG antibodies. Clinical data were obtained from all the study subjects Comorbidities (arterial hypertension, diabetes, obesity, and cancer), initial symptoms were recorded and the evolution regarding the severity of COVID-19 was recorded as the need for hospitalization, intensive care unit (ICU) and mechanical ventilation. The follow up was carried out through medical consultation with an appointment every month that included direct interrogation, examination, and peripheral blood collection for the IgG quantification. The detection of antibodies was carried out in peripheral blood using chemiluminescence microparticle immunoassay.
Results: 237 patients with positive PCR for SARS-COV-2 were included, of which 147 presented IgG antibodies (62%), 112 (76%) without comorbidity and 35 (24%) with

comorbidities, by the sixth month only 2.7% continue with positive antibody measurements. Patients with comorbidities reach higher IgG levels than patients without comorbidities the basal titters were: 5.17 for patients without comorbidities vs 6.96 for the group with comorbidities ($p<0.001$). Conclusion: We found an association between the presence of comorbidities and high levels of IgG units in pediatric patients with COVID-19. Additionally, patients with more severe course of the disease have higher levels of IgG and by the third month less than 35% have immunity

Comment 3: the introduction of the main text did not have comments on the clinical needs and significance of the focus on the longitudinal changes in pediatric patients with COVID-19 and the clinical needs for the focus on the subgroups with comorbidities.

Reply: 3: thank you very much for your observation; we made some changes in the introduction.

Changes in the text:

We add 2 comments

- in lines 116-127: The S protein includes S1 and S2 subunits that aid in host infection, while the N protein plays an important role in the transcription and replication of viral RNA (7). The presence of IgM, IgG and IgA, may be related to disease severity and some studies in adult population have associated that that IgA and IgG levels were markedly higher in patients with severe disease compared with patients with mild or moderate disease (7), It has also been reported that patients with comorbidities present with cases of more severe infection(8), however, longitudinal follow-up studies evaluating the dynamics of humoral response in the pediatric population are scarce. There are studies published in the pediatric population that compare the behavior of the humoral response by the severity of the infection (9), however, it is not described in relation to the presence of comorbidities and the severity of the infection.
- In lines 138-141: and the role of antibody persistence in the development of long-lasting protection against the virus is controversial. However, to understand long-lasting immunity after the infection, a proper characterization of humoral immune response is required

Comment 4: the methodology of the main text needs to accurately describe the clinical research design, sample size estimation, diagnosis of comorbidities, assessment of other clinical characteristics such as severity of COVID-19, and follow up procedures. The statistics needs to describe the handling of missing data and ensure $P<0.05$ is two-sided.

Reply 4: thank you very much for your observation; we made some changes in the methodology to be more specific.

Changes in the text:

- In line 146 we add: A prospective comparative cohort study was carried out
- In lines 170-183: we change the drafting: The study included all the subjects with a history of symptoms or who have been in contact with a confirmed case of SARS Cov-2) from 1 month to 17 years 11 months with a positive RT-PCR confirmatory test for SARS-CoV-2, a documented follow-up of the disease's evolution and measurement of IgG antibodies, who had confirmed their participation by signing an informed consent form. Clinical data were obtained from all the study subjects and the convalescent stage was confirmed, defined as the absence of symptoms for more than 15 days in mild cases, and with negative RT-PCR test results for SARS-CoV-2. Comorbidities (arterial hypertension, diabetes, obesity, and cancer), initial symptoms were recorded and the evolution regarding the severity of COVID-19 was recorded as the need for hospitalisation, intensive care unit (ICU) and mechanical ventilation. The follow up was carried out through medical consutaltion with an appointment every month that included direct interrogation, examination and priphereal bloth collection for the IgG quantification.
- I lines 218-219: we add the next sentence en relation of the question of missing data: For the patients that did not express IgG the result was reported in the analysis as 0.
- In lines 221-237: we change the drafting: Qualitative variables were expressed in frequencies and percentages and quantitative variables in medians and interquartile ranges (Kolmogorov–Smirnov test demonstrated non-parametric behaviour of the variables). A comparison test was performed using Chi-squared test and Fisher's exact test. The Mann–Whitney U-test was used to measure the quantitative variables.

Reviewer B

The study describes IgG antibody responses after SARS-CoV-2 infection in pediatric patients with or without comorbidities. The results indicate that six months after SARS-CoV-2 infection IgG antibodies are lost in the majority of infected children. The topic is interesting with respect to the immune protection children develop against reinfection, the design of the study is straight forward, but the presentation of the results should be improved.

I have the following comments:

Comment 1: it is not clear whether IgG levels in Figures 1 to 3 represent antibodies that are directed against spike, nucleocapsid or both antigens. This makes a big diference with respect to protection against reinfection. Where the IgG antibodies presented in the Figures measured by ELISA or by CMIA ?

Reply 1: Thank you very much for the comment, we made some changes in the methodology and delete the part of the measure by ELISA, so CMIA is the only method that we used for the detection of antibodies against the SARS-CoV-2 nucleocapsid protein and we clarify in the figures 1 to 3.

Changes in the text:

- We delete the comment of the quantification by ELISA in methodology.

- We add de legend of that the antibodies in figure 1-3 are IgG against the SARS-CoV-2 nucleocapsid protein.

Comment 2: What is the unit of measurement in Figures 1 and 3 ? Are there median or mean values depicted in Figure 3, and why are measures of distribution (SD or IQR) missing ? A considerable proportion of the children studied required hospital treatment for SARS-CoV-2 infection. It would be interesting to know if IgG antibody responses are different after hospitalization i.e. more severe COVID-19 disease. A comparison to a commonly used unit of measurement for anti-SARS-CoV-2 IgG is neede, e.g. BAU/ml as presented in the WHO standard NIBSC 20-136.

Reply 2: thank you for the observations, we add the measure of distribution in the figures and we add a new analysis by severity of the COVID-19.

Changes in the text: in lines 271-275: In table 3 we present the differences between the severity of COVID-19, the zeros represent that the patients did no express IgG against SARS-CoV-2 nucleocapsid protein. Patients with severe COVID-19 have more humoral response with the produccion of more IgG that patients with mild COVID-19 (p=0.001)

Comment 3: The percentage numbers given in Table 1 should be checked, as the sum of percentages with and without comorbidity does not result in 100%, and the percentages do not match the N, e.g. 17/46 is not 48.5%

Reply 3: thank you for noticing, we corrected the typographical error

Changes in the text: We corrected the percentage error in table 1

Comment 4: the discussion is much to long and should focus on the results reported and the comparison to other studies and/or the IgG response after adult SARS-CoV-2 infection. Discussion of vaccine responses should be omitted, as this is not a vaccination study.

Reply 4: thank you for the observation, we delete the part of vaccines in the discussion

Changes in the text: we delete this commnents: According to Uprichard et al. the two SARS-CoV-2 mRNA vaccines, BNT162b2 (Pfizer–BioNTech) and mRNA-1273 (Moderna), induce robust anti-body responses COVID-19 vaccine. Vaccination against COVID-19 in the pediatric population is far behind compared to the adult population worldwide, although in February and March 2022 the application of BNT162b2 (Pfizer–BioNTech) and mRNA- 1273 (Moderna) respectively was approved in patients aged 6 months to 5 years in the United States, in other countries of the world, vaccination has not even been started in children aged 6 to 12 years, nor are pediatric patients with comorbidities being considered to start with the vaccination schedule, a different situation from the adult population since patients with comorbidities had preference to receive their vaccine, we demonstrate that after six months

most of the patients are negative for Anti-SARS-CoV-2 IgG Antibodies, so it becomes imperative to have the support of the vaccine in this population who were not initially prioritized in 2021 for COVID-19 vaccination. Besides in adults it reported that in the absence of immunization, approximately 14% of adult patients will present with dyspnea or require intensive care (although this varies according to the characteristics of the population). 5% of patients become critically ill with an additional hospital stay of two to three weeks (median 16 days).